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Role of the microbiome, probiotics, and 'dysbiosis therapy' in critical illness

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Purpose of review
Loss of 'health-promoting' microbes and overgrowth of pathogenic bacteria (dysbiosis) in ICU is believed to contribute to nosocomial infections, sepsis, and organ failure (multiple organ dysfunction syndrome). This review discusses new understanding of ICU dysbiosis, new data for probiotics and fecal transplantation in ICU, and new data characterizing the ICU microbiome.

Recent findings
ICU dysbiosis results from many factors, including ubiquitous antibiotic use and overuse. Despite advances in antibiotic therapy, infections and mortality from often multidrug-resistant organisms (i.e., *Clostridium difficile*) are increasing. This raises the question of whether restoration of a healthy microbiome via probiotics or other 'dysbiosis therapies' would be an optimal alternative, or parallel treatment option, to antibiotics. Recent clinical data demonstrate probiotics can reduce ICU infections and probiotics or fecal microbial transplant (FMT) can treat *Clostridium difficile*. This contributes to recommendations that probiotics should be considered to prevent infection in ICU. Unfortunately, significant clinical variability limits the strength of current recommendations and further large clinical trials of probiotics and FMT are needed. Before larger trials of 'dysbiosis therapy' can be thoughtfully undertaken, further characterization of ICU dysbiosis is needed. To addressing this, we conducted an initial analysis demonstrating a rapid and marked change from a 'healthy' microbiome to an often pathogen-dominant microbiota (dysbiosis) in a broad ICU population.

Summary
A growing body of evidence suggests critical illness and ubiquitous antibiotic use leads to ICU dysbiosis that is associated with increased ICU infection, sepsis, and multiple organ dysfunction syndrome. Probiotics and FMT show promise as ICU therapies for infection. We hope future-targeted therapies using microbiome signatures can be developed to correct 'illness-promoting' dysbiosis to restore a healthy microbiome post-ICU to improve patient outcomes.

Keywords
antibiotics, bacteria, critical care, fecal transplant, infection

INTRODUCTION: ROLE OF THE MICROBIOME IN HEALTH AND ILLNESS

One of the most exciting scientific advances in recent years has been the realization that commensal microorganisms (our microbiome) play key roles in our physiology, including protection against infection, in drug metabolism, vitamin synthesis, nutrition, as well as in response to disease. A surprising finding is that disruption of the homeostasis of the microbiota, known as 'dysbiosis,' may be as vital as host genetics in the development of a range of diseases, such as inflammatory bowel disease, obesity, diabetes, and cardiovascular disease. This suggests that it may be possible to monitor, prevent, or even cure human disease through regulating the human microbiota. Recent advances in culture-independent microbiome DNA sequencing methods, even in just the last few years, have resulted in an unprecedented growth in our understanding of this vital and dynamic organ. The medical community has put a large emphasis in

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KEY POINTS

- Loss of 'health-promoting' microbes and overgrowth of pathogenic bacteria (dysbiosis) in ICU is believed to contribute to nosocomial infections, sepsis, and organ failure (multiple organ dysfunction syndrome).
- Recent data indicate as many as 37% of hospital antibiotic prescriptions are unnecessary or inappropriately prescribed, further incidence and mortality from (often antibiotic-resistant) infections like *C. difficile* are rising rapidly and optimal alternatives to antibiotics to treat infection are needed.
- Probiotics show promise to reduce ICU infections and fecal microbiota transplantation is demonstrating efficacy to reduce *C. difficile* colitis; however larger, targeted trials are needed to define how and who to best implement 'dybiosis-therapy' in.
- Prior to widespread implementation and large clinical trials of 'dysbiosis therapy,' a greater understanding and characterization of the microbiome changes in the ICU using culture-independent, amplicon and metagenomic-based sequencing techniques are needed in broader ICU populations.
- New initial data from our ongoing ICU microbiome research reveal a rapid and marked change from a 'healthy' microbiome to disrupted microbiota (dysbiosis) in a broader ICU population.

the eradication of microbial life, and in many cases for good reason. But, perhaps, we should instead consider how to preserve or reestablish a 'health-promoting' microbiome during and after critical illness through targeted interventions, such as probiotics, prebiotics, fecal transplants, and or even synthetic 'stool pills' to improve outcome in critical illness.

IS OUR CURRENT APPROACH TO INFECTION WORKING?

A great deal of time and effort is spent in eradicating bacteria and other microbial, fungal, and viral species in the ICU. The US Centers for Disease Control reveal 55% of all hospitalized patients receive an antibiotic during their stay, and in the ICU, this number increases to ~70% of patients. This observation was confirmed by a recent multinational ICU study of more than 14 000 patients in over 1200 ICUs which found that 51% of patients were considered to be infected on day of survey and a striking 71% were receiving antibiotics [1]. The hospital mortality rate of infected patients was found to be more than twice that of noninfected patients (33 vs. 15%). Clearly, infection and sepsis remain a major driver of morbidity and mortality despite advances in hospital care and widespread ICU use antimicrobial therapy [1]. As recently described by Singer and Gynne [2], it is likely that this antibiotic use has in part contributed to an impressive 22-fold fall in crude mortality rates for infectious diseases in the United States between 1900 and 1980. Yet, it is troubling that mortality rates from infectious disease (up to 1996) increased – by 50% – with the septicemia rate nearly doubling [2]. It remains unclear if the earlier reductions in mortality and increased life expectancy were due primarily to antibiotics innovations, or more likely, because of improved public health and education.

The massive global reliance on antibiotic use comes at great financial expense with antibiotics accounting for up to 30% of a hospital's drug budget [3]. Unfortunately, the potential risk for patients far exceeds the financial costs. Evidence suggests that as many as 37% of antibiotic regimens are unnecessary or not compliant with guidelines [4▪▪]. This inappropriate antibiotic use leads to the emergence of multidrug resistant bacterial infections; the incidence of these infections is rising rapidly both in the United States and worldwide [5▪▪]. A recent *New England Journal of Medicine* article estimates antibiotic-resistant *Clostridium difficile* occurs now in more than 450 000 patients per year in the United States alone [5▪▪]. Additionally, these multidrug-resistant infections are also becoming increasingly lethal. For example, *C. difficile* is estimated to contribute to ~30 000 deaths/year in the United States [5▪▪,6]. Further, the US Centers for Disease Control indicates 'death rates from sepsis following infections (like *C. difficile*) have increased at a rate greater than any other common cause of mortality in the last year for which data were available' [7]. And as stated, this is punctuated by mortality rates from infectious disease in general (up to 1996) increasing by 50%, again with the septicemia rate nearly doubling [2]. Thus, more advanced antibiotics do not appear to be translating to increased survival from infectious disease, but instead to increasingly aggressive resistant organisms and emergence of increasingly lethal pathogens like *C. difficile*. 'Is it possible we need to rethink our strategy toward microbial therapy in the ICU?'

ANTIBIOTICS KILL MORE THEN JUST PATHOGENS

These concerns around antibiotics are compounded by the fact that antibiotics currently used to attempt to treat infection not only kill pathogens but also 'health-promoting' microbes. These adverse effects include the hypothesized loss of commensal gastrointestinal microbiota, which enables overgrowth of

unwanted organisms (dysbiosis). This may have significant implications for organs far outside the gastrointestinal tract as well. The gut has long been described as the 'motor' of systemic inflammatory response syndrome and of organ failure regardless of the location of the initial infection [8▪▪]. Thus, the effect of alterations in the gut microbiota and gut barrier homeostasis are thought to be transmitted to and propagated by downstream organs, such as the spleen and lung where large immune cell populations are harbored [8▪▪,9,10], leading to inflammation-induced organ failure in the ICU.

At the cellular level, organ failure that ultimately leads to death in the ICU has long been attributed to mitochondrial failure. It has long been known that mitochondria trace their evolution from bacteria that produce energy for our cells. Recent literature not surprisingly reveals that mitochondria are known to be damaged by many of the antibiotics we commonly administer in the ICU [2]. Thus, we and others hypothesize that antibiotics may be contributing to organ failure by not only leading to dysbiosis but also by damaging the very core of our cells' energy production [2].

IS THERE ANOTHER WAY TO PREVENT AND TREAT INFECTIONS?

'Is it possible we should be giving ''friendly'' microbes to our patients, not eradicating them to improve outcome?' For young Kaitlin Hunter, microbes were the best friends she could ever have. As described by national news reports (http://www.cnn.com/2012/09/26/health/fecal-transplant/) in 2011, when Kaitlin was only 20 years old, she was critically injured in a motor vehicle accident. She recovered from her injuries after just a month of hospitalization, but then as she neared returning home she was stricken with severe, life-threatening *C. difficile* colitis. Nine courses of antibiotics failed to treat the aggressive infection. In desperation, her physicians then turned to a novel idea: rather than treating the infection with more antibiotics why not give more bacteria in the form of a stool transplant from her mother. Almost immediately, Kaitlin's *C. difficile* was cured and never returned. Was this a miracle or should we have known this would be successful from the start? We know now that this was far from an uncommon miracle as recent data indicate that fecal transplantation has a more than 90% cure rate in even the most resistant *C. difficile* colitis cases [11▪]. How is it possible that using stool as therapy, what most would consider unsanitary at best (unethical at worst), is so effective?

The answer to Kaitlin's 'miracle cure' may lie in new insights from the study of the microbiome, which is beginning to call into question what it means to be 'human.' Perhaps, our success as a species is reliant on the trillions of bacterial symbiotes we harbor and depend on. It is first important to recognize that we live in a world dominated by bacteria [12▪]. Multicellular lineages like humans are rare with most of the molecular diversity of life residing in microbes. Further, what is it that truly makes us human? Many of us believe it is our cells that make us human; however, as shown in Fig. 1, new data show we are made up of as many bacterial cells as human cells (about a 1 : 1 ratio) [13▪]. Many would believe it is our genes that make us human; however, there are ~20 000 human genes and an estimated 2–20 million microbial genes, making us about 1% human and 99% bacterial based on our genes alone. Again, this raises the key question that to fight infection organ failure should we be increasingly focused on treating dysbiosis by restoring a normal, healthy microbiome in our ICU patients, as it makes up as much or more of our bodies in health than our own cells or DNA?

THE EVOLVING ROLE FOR PROBIOTICS AND FECAL TRANSPLANTATION TO 'RESTORE HEALTH' IN ILLNESS

More objectively, (as suggested by Loupazone *et al.* [14]), perhaps we need to 'resod' the lawn that is

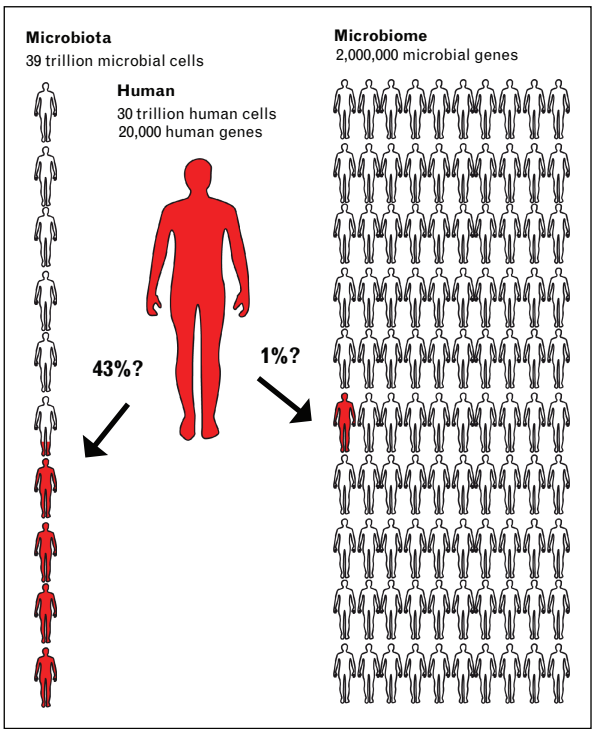

FIGURE 1. Ratio of microbial to human cells and genes in human body.

blighted by critical illness and antibiotics with probiotics, fecal transplants, and even 'stool pills' (see Fig. 2)? This concept is supported at a mechanistic level as described in a number of very recent and comprehensive review articles [8▪▪,15▪▪]. Alterations in intestinal homeostasis and gut microbiota in critical illness have been associated with increased inflammatory cytokine production, gut barrier dysfunction, and increased cellular apoptosis all of which can contribute to multiple organ failure. To modulate this 'motor' of systemic inflammation, it has been hypothesized that repletion of health-promoting bacteria via probiotics, prebiotics, stool transplantation, or combination therapies may be a promising intervention to maintain gut integrity and prevent pathologic alterations in the gut (and other body sites) microbiota or 'dysbiosis' [16▪,17▪,18▪▪]. Beneficial effects of probiotic interventions have been shown to include induction of host cell antimicrobial peptides, release of antimicrobial factors, suppression of the immune cell proliferation, stimulation of mucus and IgA production, antioxidative activity, inhibition of epithelial cell nuclear factor κ-B activation, prevention of gut apoptosis, and other epithelial barrier protective effects [8▪▪,19▪,20]. As a result, a growing number of clinical trials utilizing probiotics, and even stool transplantation, in critical illness are being conducted.

REVIEW OF CURRENT DATA FOR PROBIOTIC USE IN CRITICAL ILLNESS

Probiotic use in critical care has recently been the subject of a number of meta-analyses focused on a range of outcomes after critical illness. A role for probiotics in reducing the risk of ICU infection was initially described by Petrof *et al.* [21] in 2012. Many clinical trials and meta-analysis efforts have focused on the role of reducing ventilator-associated pneumonia (VAP). In spite of promising data for probiotic use in reducing overall infections, the role of probiotics as a strategy to preventing VAP has been controversial. In 2010, Siempos *et al.* [22] aggregated five probiotic trials demonstrating a reduction in the incidence of VAP and subsequently, Barraud *et al.* [23] also showed a beneficial effect on ICU-acquired pneumonia. However, in 2012, Petrof *et al.* [21] and Wang *et al.* [24] were unable to demonstrate a significant effect of probiotics therapy on VAP. More recently, a Cochrane review of probiotic therapy specifically for VAP [25▪], found with a low quality of evidence that probiotic therapy is associated with a reduction in the incidence of VAP. In 2015, an updated meta-analysis from the Canadian Clinical Practice Guidelines Committee became available on www.criticalcarenutriton.com. This analysis evaluated 28 studies of probiotic therapy in critical care published up to late 2014. The new results indicate that probiotics continue to show a significant reduction in infections following critical illness [relative risk 0.82, 95% confidence interval (CI) 0.69, 0.97, $P=0.02$, heterogeneity $I2=41\%$] (see Fig. 3) and a trend to reduction in VAP (relative risk 0.74, 95% CI 0.55, 1.01, $P=0.06$, heterogeneity $I2=45\%$). A trend toward a decrease in ICU length of stay when results of 14 trials were pooled (weighted mean difference −3.26, 95% CI −7.82, 1.31, $P=0.16$, heterogeneity $I2=93\%$) although significant statistical heterogeneity was present in these data. No significant effect on mortality, hospital length of stay, or other outcomes was noted. The group noted that these estimates were found to be sensitive to the quality of the primary trials. This reduction in infections disappeared when only high-quality studies were considered. Further, the potential for statistical and clinical heterogeneity indicated that further trials were needed. Based on this analysis, the Canadian Clinical Practice Guidelines Committee concluded that 'the use of probiotics should be considered in the critically ill patients.' As a wide range of probiotic species and doses were utilized in these trials, no recommendation could be made for the dose or a particular type of probiotic, with the exception of *Saccharomyces boulardii*, which should not be used as it is considered unsafe in ICU patients. 'However, further large and well designed clinical trials are needed to strengthen the recommendation of probiotic use and to confirm these benefits in critical illness'.

Very recently, a number of new trials of probiotic therapy in critical care have been published [26▪,27▪▪] not included in the recent unpublished Canadian Guidelines Meta-Analysis. These include a randomized, controlled multicenter trial by Zeng

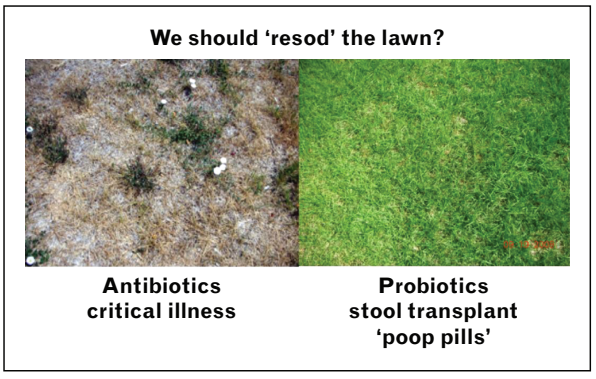

FIGURE 2. Role of 'dybiosis therapy' to restore 'health-promoting' microbiome in critical illness.

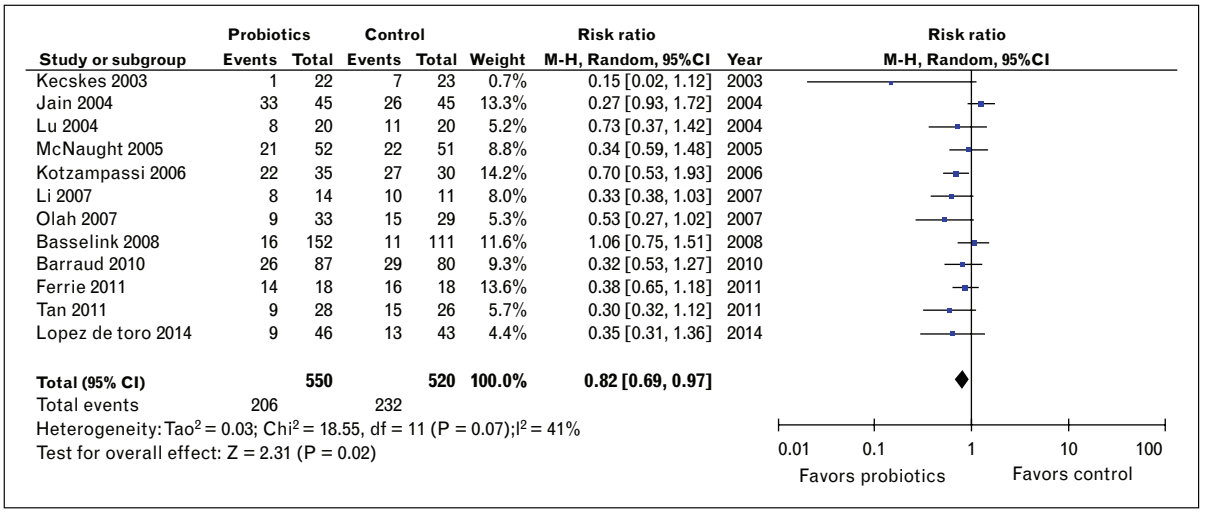

FIGURE 3. Effect of probiotic therapy on infection in ICU.

et al. [27▪▪] involving 235 critically ill adult patients expected to receive mechanical ventilation for at least 48 h. Patients were randomized to receive either a probiotics capsule containing live *Bacillus subtilis* and *Enterococcus faecalis* (Medilac-S), 0.5 g three times daily through a nasogastric feeding tube plus standard preventive strategies or standard preventive strategies alone, for a maximum of 14 days. The results revealed the incidence of microbiologically confirmed VAP in the probiotics group was significantly lower than in control patients (36.4 vs. 50.4%, respectively; $P = 0.031$). Further, the mean time to develop VAP was significantly longer in the probiotics group vs. control (10.4 vs. 7.5 days, respectively; $P = 0.022$). Future comprehensive meta-analysis systematic reviews including these newer trials are needed.

Focusing on antibiotic-associated diarrhea, a recent meta-analysis in *JAMA* showed in 63 studies and more than 11 800 patients that probiotics could reduce antibiotic-associated diarrhea by 40% [28]. Finally, a recent Cochrane meta-analysis of probiotic use in *C. difficile* colitis and diarrhea demonstrated that probiotics could reduce *C. difficile*-associated diarrhea by 64% in patients taking antibiotics (23 studies, $n = 4213$) [29▪▪]. Probiotics also reduced the risk of side-effects associated with antibiotic use in this analysis.

In addition to probiotic use, fecal transplantation has shown a more than 90% effectiveness in inducing a cure against *C. difficile* colitis [11▪,30] and 'stool pills' also may soon show promise for treating this increasingly aggressive infection. Finally, a recent case report of successful treatment of refractory severe sepsis and diarrhea with fecal transplant has been described [20]. Clinicaltrials.gov currently lists 157 clinical trials that are planned/completed or underway utilizing fecal transplantation (clinicaltrials.gov). Thus, much more data will soon be available regarding the efficacy of this therapy in multiple conditions.

CAN WE DEFINE THE ICU MICROBIOME (DYSBIOSIS) AND BETTER TARGET OUR THERAPY?

As stated, critical illness has been hypothesized to associate with loss of normal, 'health-promoting' commensal bacteria (dysbiosis), which might lead to a high susceptibility to hospital-acquired infections. Thus, a trial with prospective monitoring of the ICU microbiome with more comprehensive, culture-independent techniques to confirm and characterize this dysbiosis is urgently needed. Characterization of ICU microbiome changes may provide first steps in development of diagnostic and therapeutic interventions using microbiome signatures. Two recent pilot trials have begun to examine this question. Zaborin *et al.* [31▪▪] described ICU microbiome collection in 14 patients. The analysis focused on four patients with a prolonged length of ICU stay who showed significant disruption of the microbial community and the gut microbiota was shown to consist of ultra-low-diversity communities of multidrug-resistant pathogenic microbes. A second recent 12 patient trial by Ojima *et al.* [32▪] demonstrated changes in Bacteroidetes and Firmicutes may be related to mortality in ICU patients undergoing fecal microbiome analysis. These initial findings have led experts and major funding bodies in the field to conclude that there is urgent need for larger, more generalizable, prospective studies that characterize the microbiome in a larger critical care population to confirm and characterize this potential dysbiosis and move toward therapeutic interventions using microbiome signatures [33▪▪].

THE ICU MICROBIOME PROJECT: CHARACTERIZING THE DYSBIOSIS OF CRITICAL ILLNESS

To this end our research group (Wischmeyer *et al.*) have begun a collaboration with the Rob Knight Lab and have recently completed enrollment in an initial multicenter clinical trial of sequential microbiome sampling from ICU patients. To characterize the ICU microbiome, we collected fecal, oral, and skin samples from 115 mixed ICU patients across four centers in the United States and Canada. Samples were collected at two time points: within 48 h of ICU admission, and ICU discharge or ICU day 10. Sample collection and processing were performed under the Earth Microbiome Project protocols. We are utilizing a large control group of previously collected healthy controls and environmental surfaces including the American Gut Project, mammalian corpse decomposition samples, childhood (Global Gut), and house surfaces. In brief, the primary control group, the American Gut Project, is the largest crowd funded citizen science project, and includes more than 5000 participants, who range broadly in dietary habits, BMI, activity levels, medications, and age. For the purposes of our ICU Microbiome trial, we included healthy controls free of chronic disease and without recent antibiotic use.

CONCLUSION

Our initial results demonstrate that, when compared with healthy American gut study participants, critical illness shows rapid and distinct changes from a 'healthy' fecal and oral microbiome (Fig. 4). Fecal ICU samples tend to have a lower relative abundance of Firmicutes and increased relative abundance of Proteobacteria [34▪]. Large depletions were observed in organisms shown to confer anti-inflammatory benefits such as *Faecalibacterium* [35], which specifically is known to produce short-chain fatty acids that are vital to the gut. Conversely, many of the taxa which increased contain well recognized pathogens such as *Enterobacter and Staphylococcus*. Ongoing analysis will assess source composition of ICU samples and examine potential relationship of changes in the ICU microbiome to clinical outcome. In summary, our initial data from the ICU Microbiome Project confirm that severe dysbiosis occurs in a broad, larger population of critically ill study participants. These data may help guide creation of targeted microbial therapies, focused on correcting potentially 'illness-promoting' dysbiosis using specific probiotics or targeted, multimicrobe 'stool pills' to restore a healthy microbiome and improve outcomes in critical illness. And

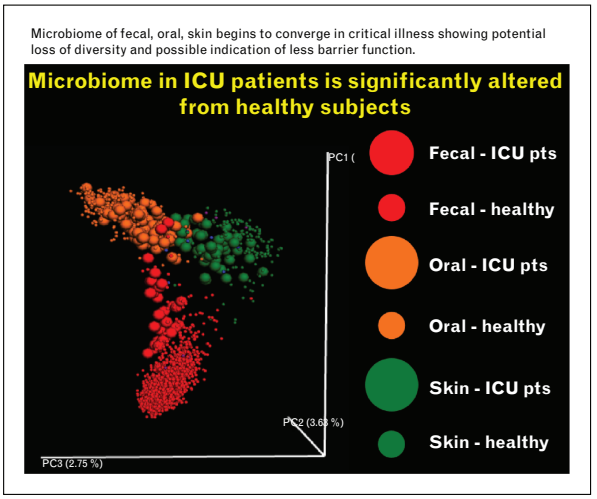

FIGURE 4. Significant alteration in microbiome in critical illness vs. health study participants. Microbiome of fecal, oral, skin begins to converge in critical illness showing potential loss of diversity and possible indication of loss of barrier function.

in the end, perhaps this is the beginning of a road to a better way to treat and prevent infection than the ubiquitous antibiotics universally given to most all patients in hospitals and ICUs today!

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None.

Conflicts of interest

There are no conflicts of interest.

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- ▪▪ of outstanding interest

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Randomized controlled trial in 235 ICU patients of probiotics capsule containing live *Bacillus subtilis* and *Enterococcus faecalis* (Medilac-S), 0.5 g three times daily vs. standard of care showing incidence of microbiologically confirmed VAP in the probiotics group was significantly lower than in control patients (36.4 vs. 50.4%, respectively; $P = 0.031$). Further, the mean time to develop VAP was significantly longer in the probiotics group vs. control (10.4 vs. 7.5 days, respectively; $P = 0.022$).

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29. Goldenberg JZ, Ma SS, Saxton JD, *et al.* Probiotics for the prevention of ■■ clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev 2013; 5:CD006095.

Meta-analysis of 23 RCTs in 4213 patients (both pediatric and adult) showing probiotics can reduce risk of *C. difficile* diarrhea by 64%. Benefits were shown in both pediatric and adult patients, and benefits were observed regardless of probiotic strain used, dose, and in both higher and lower quality studies.

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31. Zaborin A, Smith D, Garfield K, *et al.* Membership and behavior of ultra-low-■■ diversity pathogen communities present in the gut of humans during prolonged critical illness. MBio 2014; 5:e01361–e1414.

Key pilot trial of fecal ICU microbiome analysis in 14 patients. Analysis focused on four patients with a prolonged length of ICU stay who showed significant disruption of the microbial community and the gut microflora was shown to consist of ultra-low-diversity communities of multidrug-resistant pathogenic microbes.

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Recent 12 patient pilot trial of fecal microbiome analysis showing changes in Bacteroidetes and Firmicutes may be related to mortality in ICU patients.

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Editorial published with [30] above and discussion of understanding of current state of ICU microbiome characterization. Authors indicate it is difficult to know how best to target the microbiome therapeutically when the understanding of this complex ecosystem is still in a relatively nascent state. Authors indicate need for larger, more generalizable, prospective studies that characterize the microbiome in a larger critical care population to confirm and characterize potential dysbiosis with the hope to move toward focused therapeutic interventions using microbiome data.

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Abstract at Clinical Nutrition Week (ASPEN Congress) describing preliminary results from ICU Microbiome Project characterization of microbiome in ICU. Shows loss of microbial diversity and dybisosis in ICU patients as compared with healthy study participants' microbiome.

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